Crystal Structure of Yeast Mitochondrial Processing Peptidase

Alexander B. Taylor[*†], Barbara S. Smith[*†], Sakae Kitada[‡], Katsuhiko Kojima[‡], Hideki Miyaura[‡], Zbyszek Otwinowski[†], Akio Ito[‡], and Johann Deisenhofer[*†]

[*]Howard Hughes Medical Institute
[†]Department of Biochemistry,University of Texas Southwestern Medical Center, Dallas, Texas 75390, U.S.A.
[‡]Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka 812-8581, Japan

Mitochondrial processing peptidase (MPP) is a metalloendopeptidase that cleaves the N-terminal signal sequences of nuclear-encoded proteins targeted for transport from the cytosol to the mitochondria. The structure of recombinant yeast MPP has been determined to 2.5 Å resolution by the multiple anomalous diffraction (MAD) method using a selenomethionine variant. MPP is a heterodimer; its α- and β-subunits are homologous to the core II and core I proteins, respectively, of the ubiquinol-cytochrome *c* oxidoreductase complex. The active site of MPP is located in a large central cavity situated between the α- and β-subunits, lined with hydrophilic amino acids, including many glutamate and aspartate residues, from both subunits. Signal peptide substrates are rich in positively charged amino acid residues; thus, the presence of negatively charged residues in the substrate-binding region may provide opportunities for the formation of stabilizing electrostatic interactions between the enzyme and its substrate. The α-subunit has a glycine-rich loop, which is highly conserved in MPP but not in core II. This loop extends toward the active site at the β-subunit and may play a role in substrate binding and/or product release. The active site includes a negatively charged pocket formed by Glu-160 and Asp-164 of the β-subunit which may accommodate a highly conserved arginine found at position –2 from the proteolytic cleavage site of substrates. Phe-77, also of the β-subunit, is positioned to accommodate aromatic and bulky hydrophobic side chains characteristic of residues at position +1. The β-subunit has a zinc-binding motif (HxxEH) which is reversed compared to the thermolysin zinc-binding motif (HExxH). A metal ion is observed bound in this site in MPP with a water molecule coordinated to it as a fourth ligand. The zinc-binding sites of MPP and thermolysin superimpose well with an RMSD of 1.55Å for four C_α atoms compared. We conclude from the comparison of the active sites of thermolysin and MPP that the model of catalysis in thermolysin can be applied to MPP.

This work was funded by the Howard Hughes Medical Institute.

Principal investigator: Johann Deisenhofer, Howard Hughes Medical Institute, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75390, U.S.A. Telephone: 214-648-5058. E-mail: Johann.Deisenhofer@UTSouthwestern.edu.